Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2374–2378, March 1992
Neurobiology

The rat *trk* protooncogene product exhibits properties characteristic of the slow nerve growth factor receptor

(neurotrophin/tyrosine kinase/nerve growth factor binding kinetics)

SUSAN O. MEAKIN, UELI SUTER, CATHERINE C. DRINKWATER, ANDREW A. WELCHER, AND ERIC M. SHOOTER*

Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401

Communicated by Louis Sokoloff, December 30, 1991 (received for review December 4, 1991)

ABSTRACT **Two distinct nerve growth factor receptor (NGFR) complexes are present on NGF-responsive cell types; these correspond to 100 kDa and 158 kDa for the fast (fNGFR) and the slow (sNGFR) NGFRs, respectively. Previous studies indicate that each complex is derived from a separate gene product and that the sNGFR contains tyrosine kinase activity. The cDNA encoding the fNGFR has previously been cloned. In this report, a rat *trk* protooncogene cDNA has been isolated from PC12 cells and Trk has been shown to bind NGF, generating a complex of 158 kDa. Characterization of NGF–Trk interactions indicates that Trk and NGF dissociate more slowly than do NGF and the fNGFR. Moreover, NGF-bound Trk is not destroyed by trypsin digestion whereas the NGF–fNGFR complex is sensitive to trypsin digestion. These observations suggest that the *trk* protooncogene product, expressed in the absence of the fNGFR, binds NGF with properties characteristic of the sNGFR, which was identified as the high-affinity NGFR on primary neurons and PC12 cells.**

Previous work has established that two types of nerve growth factor receptors (NGFRs; type I and type II) can be distinguished on neurons of the peripheral nervous system (1, 2) and on NGF-responsive cell lines such as the rat pheochromocytoma cell line PC12 (3). More recent evidence indicates that both NGFR populations are also present in the central nervous system (4). Type I NGFRs are thought to mediate the biological responses of neuronal survival and neurite outgrowth (5, 6), whereas the role(s) of type II NGFRs is less clear.

The most distinguishing feature of the NGFR subtypes is the difference in their rates of NGF dissociation. Kinetic measurements indicate that the rate of NGF dissociation from type I NGFRs is considerably slower than from type II NGFRs (1–3). Thus, type I NGFRs are referred to as slow NGFRs (sNGFRs) and type II NGFRs are referred to as fast NGFRs (fNGFRs). Furthermore, the sNGFR and fNGFR are distinguishable on the basis of their trypsin sensitivity properties: The NGF–fNGFR complex is destroyed readily by trypsin, whereas the NGF-occupied sNGFR is not (3, 7).

Steady-state binding has also been used to distinguish between NGFR subtypes. Equilibrium binding of NGF to primary sensory and sympathetic neurons generates a biphasic Scatchard curve. The equilibrium binding constant (K_d) of the fNGFR is estimated at 1 nM and the K_d of the sNGFR is estimated at 10 pM (1, 2). Therefore, the two NGFRs have also been referred to as low- and high-affinity receptors, respectively. On PC12 cells, however, a range of equilibrium binding constants has been described, from 0.1 to 10 nM for the fNGFR and from 0.01 to 0.1 nM for the sNGFR (3, 8, 9). The differences in measured K_d of sNGFRs and fNGFRs on PC12 cells compared to sensory and sympathetic neurons is not clearly understood but may be a function of different receptor-associated factors or of experimental technique (10).

Cross-linking of ^{125}I-labeled NGF (^{125}I-NGF) to primary sensory neurons or PC12 cells identifies two major complexes that correspond to 100 kDa for the fNGFR and 158 kDa for the sNGFR (11). cDNAs encoding the fNGFR have been cloned from several species (12, 13) and evidence has been presented suggesting that the sNGFR contains the fNGFR and an additional protein (8, 14). Antibodies to the fNGFR, however, immunoprecipitate ^{125}I-NGF cross-linked fNGFR but not the sNGFR complex (15). Furthermore, the sNGFR, but not the fNGFR, can be immunoprecipitated with antibodies to phosphotyrosine (15). These results suggested a molecular independence of the NGFR complexes and suggested that the sNGFR was possibly a tyrosine kinase receptor. A tyrosine kinase activity was found specifically associated with the sNGFR, and a polypeptide of 130–135 kDa was phosphorylated in sNGFR immunoprecipitates obtained from PC12 cells (16). These data correlate with the observation that the 158-kDa complex of PC12 cells is comprised of NGF cross-linked to a polypeptide of 130–135 kDa (17) and is in line with a report by Kaplan *et al.* (18) that NGF induces the tyrosine phosphorylation and tyrosine kinase activity of the *trk* protooncogene.

Klein *et al.* (19) have demonstrated that the human Trk protein binds NGF when expressed in stably transfected NIH 3T3 cells or in Sf9 insect cells. Two classes of binding sites were observed. The major class had a K_d of 1–10 nM but was not immunoprecipitated by polyclonal antibodies (9) raised against the extracellular domain of the rat fNGFR, whereas the other class had a K_d of 0.1–0.3 nM at 4°C or 35 pM at 37°C. On this basis, it was proposed that human Trk was the high-affinity NGFR. Kaplan *et al.* (20) also reported that an engineered mouse–rat Trk fusion protein could act as a receptor for NGF, and Hempstead *et al.* (21) demonstrated that this chimeric mouse–rat Trk protein displayed a K_d comparable to the fNGFR (1 nM) in membrane preparations of transfected COS cells. In their expression system, coexpression of the human fNGFR and the Trk chimera generated a biphasic Scatchard plot with two apparent equilibrium binding constants of 1 nM and 10 pM suggesting, in contrast to the results of Klein *et al.* (19), that both receptors are necessary for high-affinity NGF binding.

To further explore the properties of Trk, we report here the cloning and sequence of the rat *trk* protooncogene† from PC12 cells and its use in transient transfection studies in COS

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NGF, nerve growth factor; ^{125}I-NGF, ^{125}I-labeled NGF; NGFR, NGF receptor; fNGFR, fast NGFR; sNGFR, slow NGFR.

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M85214).

cells. We also show that the level of Trk, like that of the fNGFR, is regulated by NGF in PC12 cells. Besides confirming that rat Trk is the 135- to 140-kDa NGF-binding protein observed in PC12 cells, we demonstrate that the rat *trk* protooncogene, if expressed in COS cells in the absence of the fNGFR, displays several NGF-binding properties ascribed to the sNGFR.

MATERIALS AND METHODS

Cell Lines. COS-7 cells were grown in Dulbecco's modified Eagle's medium containing 10% (vol/vol) calf serum. PC12 cells were cultured as described (15).

PC12 Library Construction and Screening. Oligo(dT)-primed PC12 cDNA was constructed from 5 μg of poly(A)$^+$ RNA, adapted with nonoverlapping *Bst*XI linkers, and subcloned in pCDM8 (22). A 600-base-pair trk probe was amplified from PC12 cDNA by PCR techniques with *Xho* I-linked degenerate oligonucleotides corresponding to amino acids 297–303 and 486–491 of human Trk (23). The resulting trk probe, designated trk5, was used to screen 2×10^5 primary clones. Eight clones were analyzed by DNA sequencing. The position of trk5 relative to the longest trk cDNA clone is shown in Fig. 1.

DNA Sequencing. The rat trk cDNA was sequenced on both strands by the dideoxynucleotide chain-termination method using synthetic oligonucleotides.

Plasmids. Recent sequence data of a rat genomic clone indicated a single-base difference to the rat fNGFR cDNA sequence, converting a second potential N-linked glycosylation site in the fNGFR cDNA, Asn-42, to Ser (S.O.M., C. Bitler, T. Iismaa, and E.M.S., data not shown). Sequencing of three PCR-derived fNGFR clones derived from PC12 RNA confirmed the genomic sequence (data not shown), suggesting that the fNGFR cDNA described by Radeke *et al.* (12) was derived from a rare transcript or that the cDNA isolated incurred a base substitution during the cloning procedures. The rat fNGFR cDNA was reconstructed by replacing Asn-42 with Ser (A. Baldwin and E.M.S., data not shown), cloned into pCDM8, and designated pCD-fNGFR(+). Similarly, the rat trk cDNA is designated pCD-TRK(+).

RNA Isolation and Northern Blot Analysis. Total RNA was isolated from naive and NGF (50 ng/ml)-stimulated PC12 cells and harvested at the times indicated. Total RNA (10 μg) was analyzed on a Northern blot using Hybond-N membranes (Amersham). The blot was probed with ^{32}P-labeled trk5 or fNGFR, washed under high stringency, and exposed to x-ray films.

Transient Expression in COS Cells. COS-7 cells were seeded at 1×10^6 cells per 100-mm dish 24 h prior to

FIG. 1. Nucleotide and deduced amino acid sequences of rat *trk*. The predicted signal peptide sequence is indicated with an open box and the transmembrane sequence is indicated with a stippled box. Extracellular cysteine residues are in boldface type and potential N-linked glycosylation sites are underlined. Characteristic intracellular kinase domain sequences are in boldface type. The sequence of the trk probe, trk5, is underlined with a bidirectional arrow.

transfection. Cells were transfected with 5 μg of the indicated plasmid, using the DEAE-dextran procedure, and assayed for receptor expression 48 h later.

NGF Cross-Linking and Immunoprecipitation. Mouse 2.5S NGF was purchased from Bioproducts for Science (Indianapolis) and radioiodinated with $Na^{125}I$ (Amersham) and lactoperoxidase (24). Cells were harvested (1×10^6 cells per ml), incubated with 1 nM ^{125}I-NGF, and cross-linked with 150 μM disuccinimidyl suberate (15). When indicated, L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin at 0.5 mg/ml (Sigma) was added for 15 min at 37°C after cross-linking. Cells were either lysed in SDS/sample buffer containing 100 mM dithiothreitol and analyzed by SDS/PAGE on a 6% polyacrylamide gel or lysed and immunoprecipitated as described (15). Immune complexes were analyzed by SDS/PAGE and visualized by autoradiography. Control rabbit IgGs, rabbit anti-NGF IgGs, and the monoclonal anti-fNGFR antibody (MC192) were prepared as described (15). The polyclonal Trk antiserum, raised against a 362-amino acid region encompassing the transmembrane and cytoplasmic domains of the human Trk receptor (25), was a gift of N. Hynes (CIBA–Geigy, Basel).

Dissociation Kinetics. Cells (1×10^6 cells per ml) were suspended in binding buffer (24) and incubated for 2 h at 0°C with 380 pM ^{125}I-NGF. Dissociation was initiated with unlabeled NGF (380 nM). Bound ^{125}I-NGF was determined by centrifugation through sucrose gradients. Nonspecific binding was measured from parallel reactions containing 380 nM unlabeled NGF during association.

RESULTS

cDNA Cloning of Rat *trk* from PC12 Cells. A PC12 cDNA expression library was constructed in pCDM8 (22) and screened with a 600-base-pair trk cDNA obtained as a PCR product from PC12 cells. The sequence of the longest trk clone (2633 base pairs) was determined (Fig. 1). The longest open reading frame of the rat trk cDNA predicts a polypeptide of 799 amino acids with the amino-terminal 34 amino acids characteristic of a eukaryotic signal peptide (26). Cleavage of the predicted signal peptide between Ala-34 and Ser-35 (Fig. 1) would generate a 765-amino acid polypeptide with a primary molecular mass of ≈84 kDa. A hydrophobicity plot of the predicted rat Trk protein identifies a membrane-spanning domain (amino acids 417–442) generating an extracellular domain of 382 amino acids and an intracellular domain of 356 residues. There are 11 potential N-linked glycosylation sites and 12 cysteine residues in the extracellular domain (Fig. 1). In the intracellular domain, the lysine at position 541 and the preceding Gly-Xaa-Gly-Xaa-Xaa-Gly sequence at positions 520–525 are predicted to be part of the ATP binding site. Furthermore, a diagnostic sequence of tyrosine kinases, HRDLATRN, is present at positions 651–658 (27).

Sequence comparison of the rat and human Trk proteins indicates an overall amino acid sequence identity of 86% (data not shown; refs. 23 and 28). The extracellular domain of rat Trk has two fewer potential N-linked glycosylation sites and the position of the majority of cysteines is conserved. Cys-168 of rat Trk may be functionally replaced in human Trk by Cys-177 (23, 28). However, Cys-302 of rat Trk is absent in human Trk without an apparent substitute. Interestingly, the latter cysteine residue is present in rat TrkB and pig TrkC at the analogous position (23, 29). In addition, the rat Trk protein contains a 6-amino acid insertion between positions 392 and 393 of the human Trk sequence, the exact location of several genomic rearrangements that have been shown to generate transforming alleles of human *trk* (30).

Expression of trk and fNGFR mRNA Is Differentially Regulated by NGF in PC12 Cells. To determine if *trk* expression is regulated by NGF treatment in responsive cell lines, RNA was isolated from naive and NGF-treated (50 ng/ml) PC12 cells (0.5 h, 6 h, 24 h, 2 days, 3 days, and 7 days after stimulation) and analyzed on a Northern blot. As shown in Fig. 2, the steady-state level of fNGFR mRNA (3.7 kilobases) appeared repressed after 0.5 h, subsequently increased steadily, was induced at day 1 (see also ref. 31), and peaked 3 days after NGF stimulation. In contrast, the level of trk mRNA (2.9 kilobases) slowly decreased up to 1 day after NGF treatment but was subsequently induced between days 2 and 3. Thus, our results suggest different regulatory mechanisms for the two receptor subtypes.

The NGF-Bound Trk Receptor Displays Trypsin Stability Comparable to the sNGFR Complex of PC12 Cells. The NGF-binding properties of rat Trk and the rat fNGFR were assayed by transient expression in COS cells. Forty-eight hours after transfection, cells were assayed by ^{125}I-NGF binding and cross-linking. Lysates were prepared and immunoprecipitated with either MC192 (specific for the fNGFR) or a polyclonal antibody to Trk. Immunoprecipitated complexes were analyzed by SDS/PAGE and compared to MC192 and immunoprecipitated Trk complexes obtained from ^{125}I-NGF cross-linked PC12 cells. As shown in Fig. 3*A*, both the fNGFR and the trk-transfected COS cells express an NGF-binding protein comparable to the immunoprecipitated 100-kDa and 158-kDa complexes obtained from PC12 cells (compare lanes 2 and 3 to lanes 5 and 6). The complex from trk-transfected COS cells migrated slightly faster than the 158-kDa complex from PC12 cells, which may reflect differential posttranslational modifications of the Trk protein in different cell types (18, 19).

Trypsin sensitivity was assayed as a first distinguishing parameter of sNGFRs and fNGFRs. Samples from ^{125}I-NGF cross-linked cells were treated with trypsin and the NGFR complexes were immunoprecipitated with anti-NGF antibodies. As shown in Fig. 3*B*, trypsin destroyed the NGF–fNGFR complex (compare lanes 3 and 4). In contrast, NGF-bound Trk was not destroyed by trypsin digestion, although the size of the complex became more heterogeneous and was reduced in molecular mass (compare lanes 5 and 6). The relative trypsin resistance of NGF-bound Trk in transfected COS cells is comparable to that of the 158-kDa complex (sNGFR) of PC12 cells (see lanes 7 and 8). Under these conditions, the NGF–fNGFR complex of PC12 cells is degraded, and the NGF–sNGFR complex is reduced in molecular mass by ≈10 kDa (11, 15).

Dissociation of NGFR Complexes. Initial cross-linking data indicated that ^{125}I-NGF was displaced rapidly from fNGFR-transfected COS cells. In contrast, ^{125}I-NGF I-NGF-binding to trk-transfected cells was relatively resistant to dissociation at 0°C (data not shown), in agreement with described properties of type I NGFRs in PC12 cells (11). Thus, we deter-

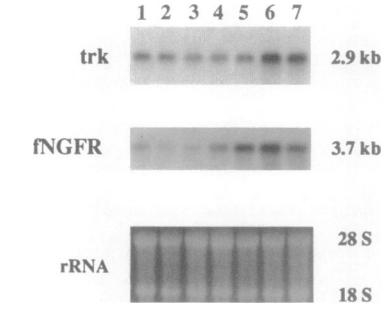

FIG. 2. NGF stimulation of trk and fNGFR mRNA in PC12 cells. Total RNA (10 μg) from NGF-stimulated PC12 cells was harvested and analyzed. rRNA was visualized by ethidium bromide staining before transfer. Times of cell harvest are as follows. Lanes: 1, 0; 2, 30 min; 3, 6 h; 4, 24 h; 5, 2 days; 6, 3 days; 7, 7 days.

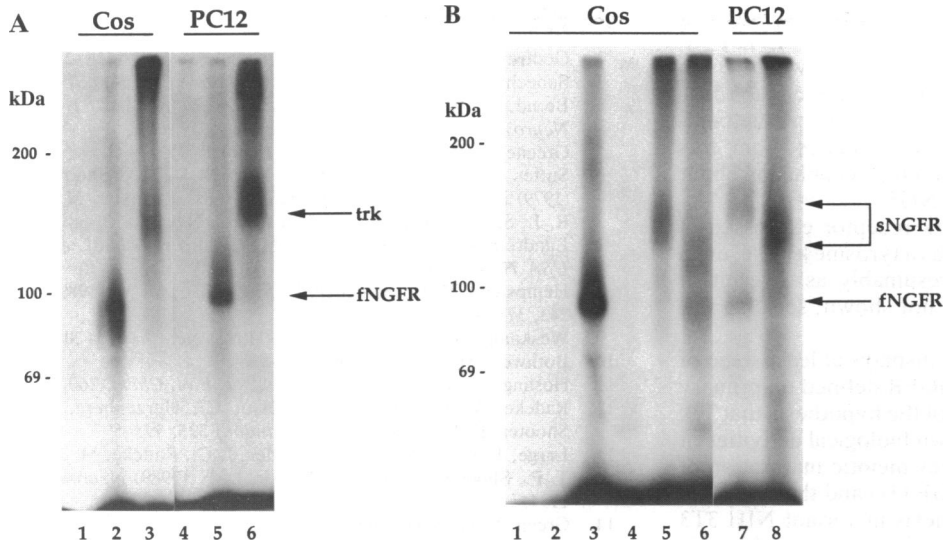

FIG. 3. (*A*) ^{125}I-NGF cross-linking of fNGFR and trk-transfected COS cells. COS cells transfected with CDM8 (lane 1), pCD-fNGFR(+) (lane 2), and pCD-Trk(+) (lane 3) were immunoprecipitated with MC192 (lanes 1 and 2) and anti-Trk antibodies (lanes 1 and 3). PC12 cells were immunoprecipitated with control IgGs (lane 4), MC192 (lane 5), and anti-Trk antibodies (lane 6). (*B*) Trypsin effects on NGF cross-linked to the fNGFR and Trk. Lanes: 1–6, COS cells transfected with CDM8 (lanes 1 and 2), pCD-fNGFR(+) (lanes 3 and 4), pCD-Trk(+) (lanes 5 and 6); 7 and 8, PC12 cells; 2, 4, 6, and 8, effects of trypsin (0.5 mg/ml) for 15 min at 37°C.

mined the dissociation kinetics of transfected NGFRs in COS cells. Transfected COS cells were labeled with ^{125}I-NGF (0.38 nM) for 2 h on ice (equilibrium binding; data not shown) and dissociation was initiated by addition of a 1000-fold excess of unlabeled NGF. The results indicate that the half-time of dissociation ($t_{1/2}$) from the fNGFR was <1 min, and the $t_{1/2}$ from Trk was considerably slower (Fig. 4). Surprisingly, the dissociation from Trk appeared to be composed of two components. The faster dissociating component had a $t_{1/2}$ of ≈10 min, and the slower component had a $t_{1/2}$ of ≈90 min. These data correlate with the slower dissociation of NGF from Trk at 0°C as measured by chemical cross-linking (data not shown) and as reported for the sNGFR (1–3). Moreover, the remaining ^{125}I-NGF bound to trk-transfected cells after 120 min of dissociation at 0°C was rapidly displaced when the reaction mixture was shifted to 37°C, indicating that this bound ^{125}I-NGF reflects specific NGF–Trk interactions rather than nonspecific COS cell binding (Fig. 4).

DISCUSSION

This report describes the isolation and characterization of a rat trk cDNA encoding a 799-amino acid tyrosine kinase receptor. The amino-terminal 34 residues of the Trk polypeptide are predicted to encode a signal peptide. Cleavage of this sequence would generate a 765-residue protein with a primary molecular mass of ≈84 kDa. Posttranslational modification, resulting in part from 11 potential N-linked glycosylation sites, generates a processed receptor molecule that migrates with an apparent molecular mass of 130–135 kDa on reducing SDS gels.

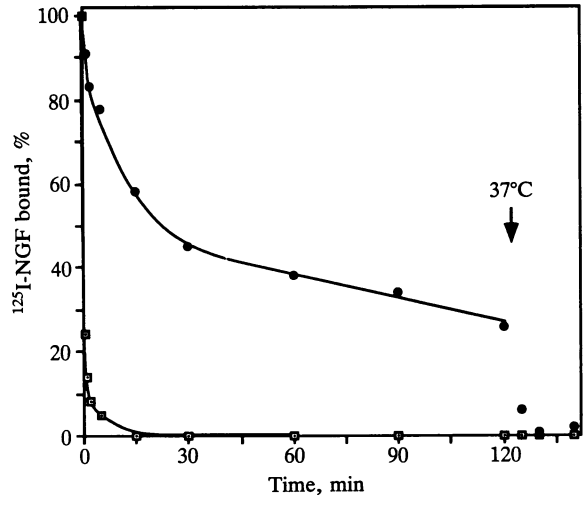

FIG. 4. Dissociation kinetics of COS-cell-transfected NGFRs. Arrow, temperature shift to 37°C; open squares, dissociation from fNGFR; solid circles, dissociation from Trk.

The amino acid sequences of rat and human Trk share an overall identity of 86%. Although the intracellular kinase domains are extremely highly conserved (94%), the extracellular sequences (that contain the putative ligand-binding domain) display only 78% amino acid identity with an incompletely conserved pattern of cysteine residues. In contrast, sequence comparison with a second member of the *trk* gene family, rat *trkB*, indicates an extracellular sequence identity of only 37% and an intracellular kinase domain identity of 75% (data not shown), comparable to sequence comparisons between rat *trkB* and human *trk* (23). These findings are of immediate interest in the light of the recent discovery that Trk binds NGF and neurotrophin 3 but not brain-derived neurotrophic factor, whereas brain-derived neurotrophic factor and neurotrophin 3, but not NGF, are ligands for the TrkB receptor (32–34).

COS-cell expression of the rat trk cDNA, and subsequent ^{125}I-NGF binding and cross-linking, generated an NGF–receptor complex comparable to the 158-kDa sNGFR complex of PC12 cells. We found that the NGF binding properties of Trk demonstrated the slow dissociation kinetics and trypsin-resistance characteristics of the sNGFR subtype. The dissociation kinetics of the fNGFR ($t_{1/2}$ < 1 min) determined in this study are in agreement with observations obtained with fNGFR-transfected L cells (12, 35), whereas the kinetics of NGF dissociation from Trk is comprised of two relatively slow components. The basis for this observation is, at present, unclear. Two important considerations should be kept in mind when comparing these data to other values present in the literature ($t_{1/2}$ of fNGFR is 5 min and NGF binding to sNGFR is stable for 30 min; refs. 3, 7, and 35). (*i*) The experiments performed here follow equilibrium binding at 0°C, whereas others (3, 7, 9) have measured dissociation after NGF binding at 37°C, a temperature at which internalization occurs. (*ii*) It remains to be determined if the fast dissociation of NGF from PC12 cells observed at 0°C is comprised solely of dissociation from the fNGFR (3, 7, 36). Interestingly, one report described the dissociation of ^{125}I-NGF from primary sensory neurons after equilibrium binding at 0°C (1). Cells treated with 10 pM NGF prior to dissociation revealed a slowly dissociating component with a $t_{1/2}$ of 20–30 min. In comparison with these values, the current data for

Trk are consistent with properties of the sNGFR subtype. It is noteworthy that the association rates of ^{125}I-NGF to trk-transfected COS cells are considerably slower than to fNGFR-transfected COS cells (15 min vs. 3 min to reach 50% of saturation binding; data not shown). However, the complex nature of the NGF dissociation kinetics from trk-transfected COS cells does not allow a final conclusion about the affinity of the Trk receptor for NGF.

Finally, we found that the rat Trk receptor expressed in COS cells was constitutively active as a tyrosine kinase, even in the absence of added NGF, presumably as a result of endogenous NGF production (data not shown; see also ref. 19).

Thus these data indicate that Trk displays at least some of the properties of the high-affinity NGFR defined on primary neurons and PC12 cells. In support of the hypothesis that Trk alone can mediate some of the known biological activities of NGF, it was shown that Trk induces meiotic maturation of *Xenopus* oocytes in response to NGF (37) and that Trk, like TrkB or TrkC, induces DNA synthesis in variant NIH 3T3 cells when stimulated by the appropriate neurotrophin (29, 34, 38, 39). With respect to the reports that both Trk and fNGFR are required for high-affinity NGF binding (21), it should be noted that ^{125}I-NGF cross-linking experiments on PC12 cells (11) do not support the hypothesis that Trk and fNGFR interact at the level of the receptors themselves. Under conditions of ^{125}I-NGF binding to sNGFR, only the ^{125}I-NGF–Trk complex is observed. If Trk and fNGFR interact to form a high-affinity binding site, a ^{125}I-NGF–fNGFR complex (and, possibly, the ternary ^{125}I-NGF–fNGFR–Trk complex) should also be formed under these conditions. Nevertheless, the fNGFR apparently modulates the tyrosine kinase activity of Trk (40), suggesting a possible interaction of the signal transduction mechanisms of the two receptors.

Kaplan *et al.* (18) reported that the level of trk mRNA in PC12 cells was not affected by NGF treatment after 2 days. This was confirmed in the present study. We found, however, that trk mRNA levels are upregulated by NGF after 3 days of treatment. This observation is in agreement with earlier studies (41) that showed that high- and low-affinity NGFRs on PC12 cells are upregulated by NGF.

The availability of the rat trk cDNA and its sequence will permit studies of the role of the Trk tyrosine kinase receptor on primary neurons, its signal transduction mechanism(s), and its role in development and neuronal regeneration in the experimentally amenable rat system. In a first step toward these goals, the data presented in this report demonstrate that the rat Trk receptor (expressed in COS cells) shows properties comparable to the sNGFR in PC12 cells, the rat cell line widely used in NGFR studies. How these findings relate to the NGFRs on sensory neurons can now be addressed.

We thank the Brutlag laboratory (Department of Biochemistry, Stanford University) for the prediction of the signal peptide sequence, G. Escher (Department of Neurobiology, Stanford University) for assistance in analyzing the Trk sequence, N. Hynes (CIBA-Geigy) for the rabbit polyclonal antibody to Trk, A. Baldwin for constructing the single glycosylated fNGFR cDNA, P. Barker for stimulating discussions, and M. J. Radeke, S. C. Feinstein, and D. R. Kaplan for communicating data prior to publication. This work was supported by grants from the National Institute of Neurological Disorders and Stroke (NS04270), the American Cancer Society (BE47J), a Damon Runyon–Walter Winchell Cancer fellowship (DRG-984) to S.O.M.; the Swiss National Science Foundation and the Swiss Academy of Medical Sciences to U.S.; a C. J. Martin Fellowship from the Australian National Health and Medical Research Council to C.C.D.; and a National Research Service Award Fellowship (NS08443) to A.A.W.

1. Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, E. M. (1979) *J. Biol. Chem.* **254,** 5972–5982.
2. Godfrey, E. W. & Shooter, E. M. (1986) *J. Neurosci.* **6,** 2543–2550.
3. Schechter, A. L. & Bothwell, M. A. (1981) *Cell* **24,** 867–874.
4. Bernd, P., Martinez, H. J., Dreyfus, C. F. & Black, I. B. (1988) *Neuroscience* **26,** 121–129.
5. Greene, L. A. (1977) *Brain Res.* **133,** 350–353.
6. Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, E. M. (1979) in *Transmembrane Signalling*, eds. Bitensky, M., Collier, R. J., Steiner, D. F. & Cox, C. F. (Liss, New York), pp. 659–667.
7. Landreth, G. E. & Shooter, E. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4751–4755.
8. Hempstead, B. L., Schleifer, L. S. & Chao, M. V. (1989) *Science* **243,** 373–375.
9. Weskamp, G. & Reichardt, L. F. (1991) *Neuron* **6,** 1–20.
10. Bothwell, M. (1991) *Cell* **65,** 915–918.
11. Hosang, M. & Shooter, E. M. (1985) *J. Biol. Chem.* **260,** 655–662.
12. Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A. & Shooter, E. M. (1987) *Nature (London)* **325,** 593–597.
13. Large, T. H., Weskamp, G., Helder, J. C., Radeke, M. J., Misko, T. P., Shooter, E. M. & Reichardt, L. F. (1989) *Neuron* **2,** 1123–1134.
14. Green, S. H. & Greene, L. A. (1986) *J. Biol. Chem.* **261,** 15316–15326.
15. Meakin, S. O. & Shooter, E. M. (1991) *Neuron* **6,** 153–163.
16. Meakin, S. O. & Shooter, E. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5862–5866.
17. Radeke, M. J. & Feinstein, S. C. (1991) *Neuron* **7,** 141–150.
18. Kaplan, D. R., Martin-Zanca, D. & Parada, L. F. (1991) *Nature (London)* **350,** 158–160.
19. Klein, R., Jing, S., Nanduri, V., O'Rourke, E. & Barbacid, M. (1991) *Cell* **65,** 189–197.
20. Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V. & Parada, L. F. (1991) *Science* **252,** 554–558.
21. Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F. & Chao, M. V. (1991) *Nature (London)* **350,** 678–683.
22. Aruffo, A. & Seed, B. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8573–8577.
23. Middlemas, D. S., Lindberg, R. A. & Hunter, T. (1991) *Mol. Cell. Biol.* **11,** 143–153.
24. Vale, R. D. & Shooter, E. M. (1985) *Methods Enzymol.* **109,** 21–39.
25. Ziemiecki, A., Muller, R. G., Xiao-Chang, F., Hynes, N. E. & Kozma, S. (1990) *EMBO J.* **9,** 191–196.
26. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
27. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241,** 42–52.
28. Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T. & Barbacid, M. (1989) *Mol. Cell. Biol.* **9,** 24–33.
29. Lamballe, F., Klein, R. & Barbacid, M. (1991) *Cell* **66,** 967–980.
30. Kozma, S. C., Redmond, S. M. S., Xiao-Chang, F., Saurer, S. M., Groner, B. & Hynes, N. E. (1988) *EMBO J.* **7,** 147–154.
31. Miller, F. D., Mathew, T. C. & Toma, J. G. (1991) *J. Cell Biol.* **112,** 303–312.
32. Squinto, S. P., Stitt, T. N., Aldrich, T. H., Davis, S., Bianco, S. M., Radziejewski, C., Glass, D. J., Masiakowski, P., Furth, M. E., Valenzuela, D. M., DiStefano, P. S. & Yancopoulos, G. D. (1991) *Cell* **65,** 885–893.
33. Soppet, D., Escandon, E., Maragos, J., Middlemas, D. S., Reid, S. W., Blair, J., Burton, L. E., Stanton, B. R., Kaplan, D. R., Hunter, T., Nikolics, K. & Parada, L. F. (1991) *Cell* **65,** 895–903.
34. Cordon-Cardo, C., Tapley, P., Jing, S., Nanduri, V., O'Rourke, E., Lamballe, F., Kovary, K., Klein, R., Jones, K. R., Reichardt, L. F. & Barbacid, M. (1991) *Cell* **66,** 173–183.
35. Rodriguez-Tebar, A., Dechant, G. & Barde, Y.-A. (1990) *Neuron* **4,** 487–492.
36. Vale, R. D., Ignatius, M. J. & Shooter, E. M. (1985) *J. Neurosci.* **5,** 2762–2770.
37. Nebreda, A. R., Martin-Zanca, D., Kaplan, D. R., Parada, L. F. & Santos, E. (1991) *Science* **252,** 558–560.
38. Glass, D. J., Nye, S. H., Hantzopoulos, P., Macchi, M. J., Squinto, S. P., Goldfarb, M. & Yancopoulos, G. D. (1991) *Cell* **66,** 405–413.
39. Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. & Barbacid, M. (1991) *Cell* **66,** 395–403.
40. Berg, M. M., Sternberg, D. W., Hempstead, B. L. & Chao, M. V. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7106–7110.
41. Bernd, P. & Greene, L. A. (1984) *J. Biol. Chem.* **259,** 15509–15516.